Effects of chemical preservative and pressing of ensiled sugar-beet pulp on the quality of fermentation process

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ABSTRACT: This study deals with effects of pressing of ensiled sugar-beet pulp and of application of a chemical preservative on the quality of fermentation process. The experimental silages had a better sensory evaluation than the control ones. In silages treated chemically with a mixture of acids, statistically significantly ($P < 0.01$) higher dry matter content, lowest pH value, the value of lactic acid and the lowest content of all acids in dry matter were found after 180 days of storage from the beginning of the experiment. The statistically significantly ($P < 0.01$) highest lactic acid content (43.39 ± 1.25 g/kg DM) was determined in the control pressed silage. The highest LA/VFA ratio (1.40 ± 0.18) was calculated for non-pressed experimental silage (D – 3 l/t of KEM). As compared with untreated control the highest percentage ($P < 0.01$) of lactic acid and of all fermentation acids was found out in silage D treated with 3 l/t of KEM (58.18 ± 0.47 g/kg DM). Undesirable butyric and propionic acids were not found in chemically treated silage samples (C, D, E, F). However, the highest ($P < 0.01$) contents of butyric acid (26.37 ± 0.91 g/DM) and propionic acid (4.58 ± 0.78 g/DM) were measured in untreated non-pressed silage samples (B). The highest ($P < 0.01$) contents of acetic acid and ethanol were found in control silage samples. The quality of these silages was evaluated as very low.

Keywords: sugar-beet pulp; silage additive; fermentation process; silage making

Both fresh and ensiled sugar-beet pulp has a high feeding value and shows a positive dietetic effect on ruminal fermentation (Rohr et al., 1986; Formigoni et al., 1993). As far as the feeding value is concerned, this feed is comparable with feeding barley (Murphy, 1986), has a favourable energy concentration and a relatively high content of fibre (Drennan, 1981). Boldt et al. (1990) compared production efficiency of fresh, dried and ensiled pulp. However, fresh sugar-beet pulp is also a suitable medium for the propagation of epiphytic microorganisms, particularly moulds, yeast and soil bacteria, which can cause a quick deterioration of this feedstuff. For a longer storage it is recommended to preserve this feed above all in the form of silage (Graf, 1981; Graf and Haluschan, 1981). Provided that the technological conditions are maintained on an optimal level, the process of ensiling of pressed sugar-beet pulp usually runs without any marked problems. Haaksma (1991) concluded that already within the first two days after ensiling, the major part of sucrose was converted to monosaccharides and organic acids, particularly lactic acid (LA) and acetic acid (AA) in amounts of 1.2–1.4% and 0.2–0.4%, resp., and that the pH-value decreased from 5.5 to 3.8. Such acidification preventively inhibited harmful bacteria, above all clostridia. Grajewski (1990) also analysed the quality of silages made of wet and pressed sugar-beet pulp. Mossakowska (1990) reported that silages made of pressed sugar-beet pulp containing 23–24% of dry matter and a sufficient amount of sucrose contained 1.18–3.50% of lactic acid and no butyric acid. On the other hand, Wyss (2002) observed that silages made of pressed sugar-beet pulp showed low levels of lactic acid. Although the pH value of silages made of sugar-beet pulp was low, differences between control and experimental silage samples were large.

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The final quality of the fermentation process of sugar-beet silage results from an interaction of endogenous and exogenous technological factors, viz. of DM content, ambient temperature and temperature of ensiled sugar-beet pulp. Rate of filling, degree of pressing, duration of fermentation process and addition of chemical preservatives can also improve the final quality of silage. When ensiling sugar-beet pulp with a lower DM content (15–18%), there is a risk of undesired fermentation associated with increased production of volatile fatty acids (VFA), especially of acetic and butyric acid and of alcohol. During the extensive fermentation of pectins, which otherwise strengthen cellular tissues and thus influence also the structure of sugar-beet pulp, high amounts of methanol, butyric acid and acetic acid are produced. The fermentation of pectin substances is always associated with deterioration of silage structure and also with high production of volatile fatty acids. This impaired pulp structure is caused partly by an increased temperature that is used for a certain period during the process of sugar extraction (Braunsteiner et al., 1983) and partly by the course of the fermentation process itself, particularly due to activities of pectinolytic clostridia (Papa and Grazia, 1987). This thermal deterioration of pulp structure may also be caused by hemicellulolytic enzymes which are produced by some yeast and lower fungi species (Kubadinov et al., 1984). To preserve the structure of ensiled sugar-beet pulp, it is recommended to use some preservatives, especially those containing organic acids (Courtin and Spoelstra, 1989). The preservative effects of formic acid, calcium formiate and acrylic acid on the quality of the fermentation process of sugar-beet pulp were investigated in our earlier study (Doležal et al., 1989). O'Kiely (1992) also concluded that the addition of 3 ml/kg of formic acid influenced the process of fermentation to a larger extent than some absorbents, e.g. rolled barley. Nonn (1987) tested a number of preservatives (formic acid, propionic acid, sorbic acid, benzoic acid, mixture of acids, nitrites and biological inoculants) in his experiments with ensiling of sugar-beet pulp with a low content of dry matter (< 20%). Hünting (2004, person. commun.) concluded that the use of preservatives containing homofermentative lactic acid bacteria did not result in a marked reduction of fermentation losses. The improvement of the fermentation process was not very distinctive because the control silage was also good. However, he observed a marked improvement of silage stability after the use of the produt Biocool (with heterofermentative lactic acid bacteria). On the other hand, Parigi-Bini et al. (1991) found out that the inoculation of pressed sugar-beet pulp silages with a commercial product containing lactobacilli (*Lactobacillus plantarum* and *Streptococcus faecium*) did not affect chemical composition and fermentation characteristics of sugar-beet pulp silage. Baldwin et al. (1986) studied the process of sugar-beet pulp ensiling with urea under model conditions and observed a rapid decrease in pH value; however, the differences in VFA contents of their laboratory silages were very small.

The aim of this study was to evaluate the effect of pressing of sugar-beet pulp and of different supplements of organic-acid preservatives on the fermentation process and on the quality of the final product.

Table 1. Statistical difference between silages groups

Variant	Dry matter (%)	pH	LA	AA
			(g/kg DM)	
A control	18.40 ± 0.12^{B,C,D,E,F}	4.1 ± 0.01^{B,C,D,E,F}	43.39 ± 1.25^{B,C,D,E,F}	72.02 ± 1.22^{B,C,D,E,F}
B control	17.81 ± 0.04^{A,C,D,E,F}	3.90 ± 0.02^{A,D,E,F}	34.99 ± 1.74^{A,C,E,F}	87.57 ± 1.40^{A,C,D,E,F}
C KEM 3 L	20.75 ± 0.03^{A,B,d,F}	3.87 ±0.02^{A,D,E,F}	29.30 ± 1.52^{A,B,D,E,F}	24.10 ± 3.35^{A,B,E,F}
D KEM 3 L	20.44 ± 0.07^{A,B,c,E}	3.67 ± 0.01^{A,B,C,F}	35.87 ± 1.32^{A,C,E,F}	25.78 ± 1.31^{A,B,E,F}
E KEM 6 L	20.78 ± 0.09^{A,B,D,f}	3.66 ± 0.01^{A,B,C,F}	2.95 ± 0.51^{A,B,C,D}	11.15 ± 0.45^{A,B,C,D}
F KEM 6 L	20.47 ± 0.11^{A,B,c,e}	3.74 ± 0.03^{A,B,C,D,E}	6.14 ± 1.94^{A,B,C,D}	13.29 ± 1.66^{A,B,C,D}

A control – pressed sugar-beet control silage, B control – non-pressed sugar-beet control silage; C – KEM 3 L – chemically 6 L – chemically treated pressed sugar-beet silage, variant 6 L; F – KEM 6 L – chemically treated non-pressed sugar-beet

^{a,b,c,d,e,f}significant differences at a significance level of 95%

MATERIAL AND METHODS

Laboratory experiments were carried out with fresh pressed sugar-beet pulp containing on average 20.93% of DM. The sugar-beet pulp was obtained from the Eastern Sugar Czech Republic, a.s., sugar refinery Němčice and ensiled in a warm condition using experimental bottles four litres in volume. The experiment was established in six replications. Model control and experimental silages were prepared in three variants, viz. with pressing (A, C, E) and without it (B, D, F). Prior to ensiling, experimental silages were uniformly treated with a preservative containing a mixture of organic acids (KEM) in the dose of either 3 or 6 litres per ton; control samples were without this additive. The preservative consisted of formic acid (55%), propionic acid (5%), ammonium formiate (24%), benzoic acid (1%) and esters of benzoic acid (1%). This preservative was manufactured and supplied by Kemira Chemical Oys (Finland). In the pressed variant both control (A) and experimental (C, E) samples were pressed to the average specific density of 722.5 kg/m^3 while in control (B) and experimental (D, F) variants of non-pressed sugar-beet pulp the average specific density was only 381.9 kg/m^3. Experimental bottles were tightly closed using Omnia twist-off caps and stored at the temperature of 27 ± 3°C for the whole experimental period. After 180 days of storage, all bottles were opened and the silage samples were analysed using standard methods to estimate some important qualitative parameters of the fermentation process that were described earlier (Doležal, 2002). The obtained results were analysed by ANOVA. Tukey's comparison procedure was applied to all treatments. Significance was declared at $P < 0.01$ and $P < 0.05$.

RESULTS AND DISCUSSION

The average DM content of fresh sugar-beet pulp was 20.93% and ranged from 20.46% to 21.88%. The ensiled material contained initially 0.5% of sucrose, i.e. only 2.39% per kg DM; this sugar content was lower than that reported by Haaksma (1991). The fresh ensiled sugar-beet pulp contained in 1 kg of dry matter 98.3 g CP, 216.4 g CF fibre, 279.2 g ADF, 502.0 g NDF and 588.2 g of N-free extract. The rumen degradability of protein was 81.08% and ME content 10.46 MJ.

Data describing the results of fermentation process in control and experimental silages after 180 day of storage are presented in Tables 1–3 and Figures 1–5. Comparing the fermentation parameters in control and experimental samples, statistically significant differences were found out. The highest content of dry matter (20.78 ± 0.09) was found in samples of pressed silage treated with the KEM preservative in the dose of 6 l/t ($P < 0.01$) while in control samples of both pressed and non-pressed silage the respective values were lower (18.40 ± 0.12 and 17.81 ± 0.04, resp.). A significantly higher ($P < 0.01$) content of DM was also found out in silage samples treated with 3 l/t of KEM. This result corresponded with data published by other authors (O'Kiely, 1992).

Differences in pH values were also statistically significant ($P < 0.01$). The lowest pH was found out in experimental silage made of pressed sugar-beet

Table 1 continued

BA	PA	SA	Ethanol	LA/VFA
(g/kg DM)				
5.27 ± 0.80^{B,C,D,E,F}	1.18 ± 0.17^{B,c,d,e,F}	121.86 ± 1.75^{B,C,D,E,F}	14.77 ± 0.60^{B,C,d}	0.56 ± 0.03^{B,C,D,E,f}
26.37 ± 0.91^{A,C,D,E,F}	4.58 ± 0.78^{A,C,D,E,F}	153.53 ± 3.13^{A,C,D,E,F}	19.64 ± 0.42^{A,C,D,e,f}	0.30 ± 0.03^{A,C,D,e,F}
0 ± 0.00^{A,B}	0 ± 0.00^{a,B}	53.40 ± 1.96^{A,B,d,E,F}	8.59 ± 0.53^{A,B,E,F}	1.22 ± 0.49^{A,B,d,E,F}
0 ± 0.00^{A,B}	0 ± 0.00^{a,B}	61.65 ± 2.05^{A,B,c,E,F}	10.93 ± 0.95^{a,B,e,F}	1.40 ± 0.18^{A,B,c,E,F}
0 ± 0.00^{A,B}	0 ± 0.00^{a,B}	14.11 ± 0.46^{A,B,C,D}	15.52 ± 2.41^{b,C,d}	0.27 ± 0.12^{A,C,D,F}
0 ± 0.00^{A,B}	0 ± 0.00^{a,B}	19.44 ± 3.51^{A,B,C,D}	15.77 ± 1.04^{b,C,D}	0.45 ± 0.19^{a,B,C,D,E}

treated pressed sugar-beet silage, variant 3L; D – KEM 3 L – chemically treated non-pressed silage, variant 3 L; E – KEM silage, variant 6 L; LA – lactic acid; AA – acetic acid; BA – butyric acid; PA – propionic acid; SA – sum of acids

^{A,B,C,D,E,F}significant differences at a significance level of 99%

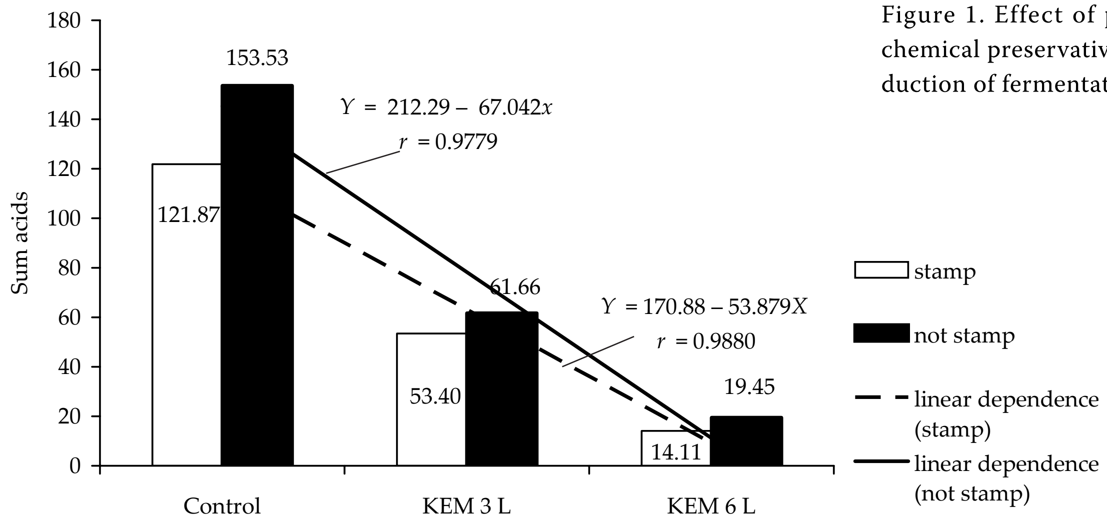

Figure 1. Effect of pressing and chemical preservative on the production of fermentation acids

pulp treated with KEM preservative in the dose of 6 l/t (3.66 ± 0.01). These data are in agreement with results published by Moore and Kennedy (1994). The application of KEM corroborated the statement published by Baintner et al. (1983), who observed that a combination of organic acids strengthened the synergic effect, decreased the pH value and improved the bactericidal effect of additives. The significantly highest pH value was found out in untreated control silage (Figure 4). Fermentation products also indicated that the course of fermentation in model silages was quite different; especially in control samples the contents of butyric acid and acetic acid were significantly ($P < 0.01$) higher. In control silages, the pressing of sugar-beet pulp showed a positive and statistically significant ($P < 0.01$) effect on the production of lactic acid. As compared with non-pressed control samples, the content of LA in experimental silages with addition of KEM preservative was lower (Figure 5). The

Table 2. Content of ethanol in pressed and non-pressed silage samples

Variant	Dry matter (%)	pH	LA	AA	BA	PA	SA	Ethanol
			(g/kg DM)					
Pressed group total	19.98	3.88	25.22	35.76	1.76	0.39	63.13	12.96
Non-pressed group total	19.58	3.78	25.67	42.22	8.79	1.53	78.21	15.45

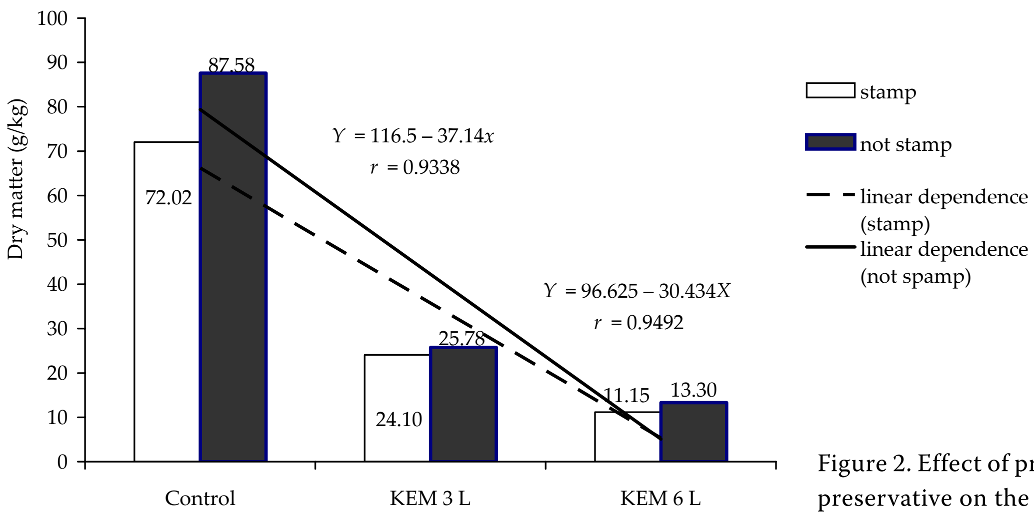

Figure 2. Effect of pressing and chemical preservative on the production of AA

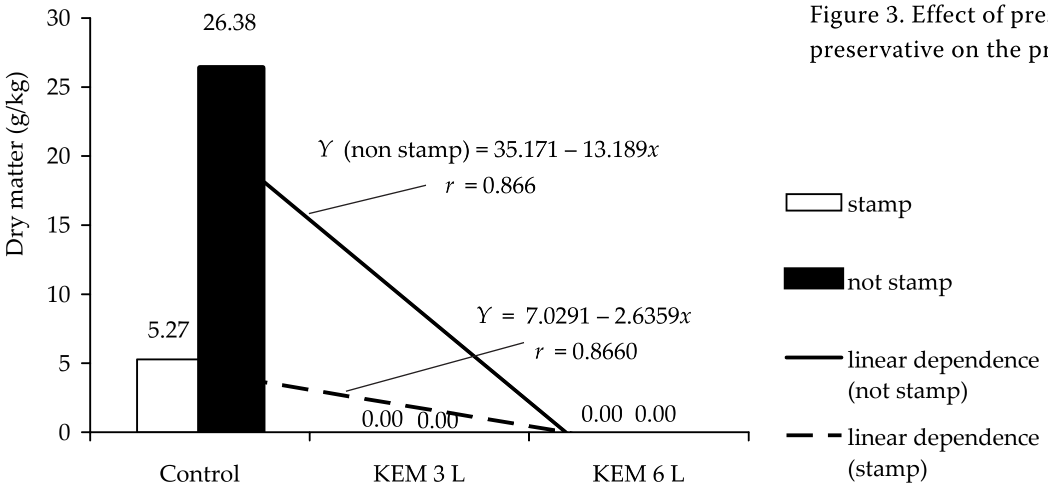

Figure 3. Effect of pressing and chemical preservative on the production of BA

control silage made of non-pressed sugar-beet pulp (F) showed significantly ($P < 0.01$) higher contents of acetic and propionic acid (Figures 2 and 3). The total sum of acids was also the highest (Figure 1). The sensory evaluation of this silage was also negative. The obtained data indicated that, as compared with (B), the factor "pressing" significantly ($P < 0.01$) reduced not only the levels of acetic, butyric and propionic acid but also the total content of fermentation acids. The control pressed silage (A) contained significantly ($P < 0.01$) less alcohol than (B). These data corroborated an earlier observation (Kalač, 1987) that the more intensive metabolisation of hemicellulose and pectins caused by fermentation of sugar-beet pulp was associated with increased production of alcohol. These data also indicated that the pectin substances were degraded in this silage. This degradation was associated not only with increased production of VFA (and particularly of butyric acid) but also with a deteriorated structure of control silages (Braunsteiner et al., 1983; Courtin and Spoelstra, 1989). In group (B) the proportion of VFA was 77.20% while in (A) only 64.40%. It seems that in silages stored at higher temperatures lactic acid can be used as a source of energy consumed by anaerobic pectinolytic bacteria. This decrease in the content of lactic acid was obvious above all in (B) where it was associated with significantly ($P < 0.01$) higher production of alcohol. A significantly ($P < 0.01$) higher LA/VFA ratio was calculated for experimental silages treated with 3 litres of the chemical preservative and its value was higher than 1.00 (1.22 and 1.40, resp.). In experimental variants treated with 6 litres of KEM per ton this ratio was significantly ($P < 0.01$) lower (0.27–0.45) obviously due to reduced production

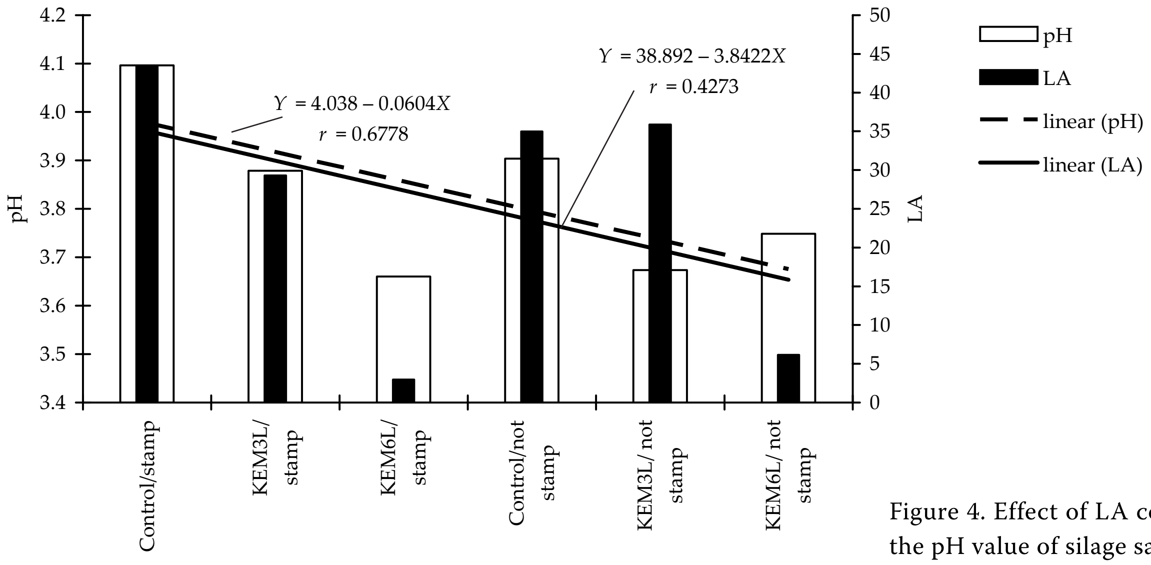

Figure 4. Effect of LA content on the pH value of silage samples

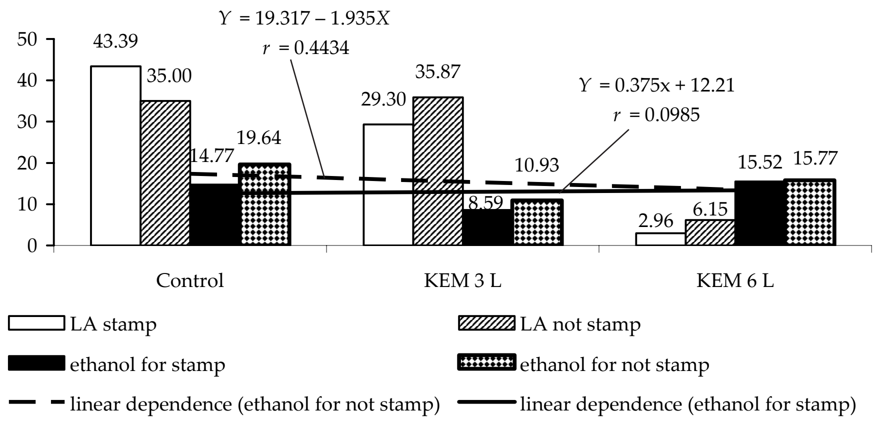

Figure 5. Relationship between the contents of LA and ethanol in ensiled sugar-beet pulp

of acetic acid. These results corroborated earlier results (Loučka et al., 1999; Doležal, 2002) and corresponded also with data published by Nonn (1987). In control silages, the LA/VFA ratio was higher in the pressed (A) variant than in the non-pressed (B) one (0.56 ± 0.03 vs. 0.30 ± 0.03, resp.). This observation indicated a direct effect of pressing on the course of fermentation. Experimental silages (C, D, E, F) with an addition of both doses of the chemical preservative (KEM) did not contain any butyric and/or propionic acid. A significant ($P < 0.01$) reduction of production of all fermentation acids in DM was found in these silages including LA and AA. A higher KEM concentration (6 l/t) caused that the amount of LA in these silage samples was significantly ($P < 0.01$) the lowest (2.95 ± 0.51 vs. 6.14 ± 1.94 g/kg DM, resp.) and accounted only for 20.91% and 31.58%, resp., of all acids. These results corresponded with data published by O'Kiely (1992) and Moore and Kennedy (1994), who observed a marked decrease in production of VFA, LA, ammonia and alcohol after the application of formic acid as an additive into the experimental silages. The regression analysis of the effects of different concentrations of chemical preservatives on the total production of fermentation acids (and/or LA) revealed a very close linear relationship between the dependent and independent variables. In pressed silages the value of *r* coefficient was high (0.9880) while in non-pressed ones in was lower (0.9779).

Lower levels of produced LA found in experimental silages were explained as resulting from inhibitive and antimicrobial effects of the tested additive, which, in a higher concentration, reduced growth and propagation of lactic acid bacteria (LAB). Results obtained after the application of this preservative corroborated also the data published by Baintner et al. (1983), who mentioned that a combination of organic acids showed a synergic effect that caused a more marked decrease in pH value and intensification of antimicrobial effects. In our study, experimental silage samples showed highly significant ($P < 0.01$) differences in pH values, which were lower than in controls and did not exceed the limit of 4.0. This decrease in pH values was caused mainly by direct acidification of all experimental silages with the chemical preservative because production of LA and all other acids was strongly reduced. This was also proved by regression analysis ($r = 0.\ 6778$) of dependent variables (pH, LA content) on the independent one (dose of preservative agent), which indicated that the increasing KEM concentration resulted, as compared with control, in marked acidification (Figure 4).

Table 3. The effect of preservative addition on the fermentation characteristics (mean of all groups)

Variant	Dry matter (%)	pH	LA	AA	BA	PA	SA	Ethanol
			(g/kg DM)					
Control	18.11	4.00	39.19	79.80	15.83	2.88	137.70	17.21
KEM 3L	20.60	3.78	32.59	24.94	0.00	0.00	57.53	9.76
KEM 6L	20.63	3.70	4.55	12.22	0.00	0.00	16.78	15.65

The effect of pressing on the decrease in pH value was not uniform (Table 2). As compared with control, the application of chemical preservative caused a statistically significant ($P < 0.01$) decrease in ethanol content ($r = 0.966$).

The content of fermentation acids and the final structure of silage indicate that an increased storage temperature showed a more negative effect on the fermentation process in control silage samples than in experimental ones. It is obvious that a higher temperature and lack of saccharides caused increased degradation of pectins; this observation corresponded with data published by Braunsteiner et al. (1983), Courtin and Spoelstra (1989) and some other authors. A higher storage temperature (> 25°C) caused that the production of VFA (particularly of AA and BA) was more intensive in control model silages; these samples also had an impaired (sometimes even slimy or greasy) consistency. This observation was in agreement with earlier data published by Braunsteiner et al. (1983). On the other hand, however, the smell of experimental samples was pleasant and their consistency was excellent. These results indicate that successful preservation of sugar-beet pulp requires not only the treatment with chemicals but also thorough pressing because even the sample of (D, F) showed moulds on its surface. Higher contents of AA, BA and alcohol in (B) indicate that the quality of fermentation process was significantly lower ($P < 0.01$) in this case than in pressed sugar-beet pulp (A).

CONCLUSIONS

The effect of pressing and addition of a chemical preservative (KEM) on the quality of fermentation process was studied in a model laboratory experiment. It is concluded on the base of results obtained that, as compared with non-pressed silage samples, the pressing of ensiled sugar-beet pulp with a higher content of dry matter (204.6 g/kg) decreased the levels of undesired butyric and propionic acids and of ethanol. The addition of different doses of a chemical preservative KEM (i.e. a mixture of organic acids) resulted in a significant ($P < 0.01$) decrease in production of total VFA, LA and pH value in experimental silage samples. Experimental, chemically treated silage samples (C, D, E, F) did not contain any butyric and/or propionic acid and their contents of ethanol were statistically significantly ($P < 0.01$) lower than in control. Model silages treated with a dose corresponding to 3 l/t showed a significantly ($P < 0.01$) higher LA/VFA ratio than control silage samples and their consistency (structure) and smell were much better.

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